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EXPERIMENTS ON THE EXTRUSION OF POLAR FILAMENTS OF CNIDOSPORIDIAN SPORES *

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It has been generally believed that when a Cnidosporidian spore is introduced into the digestive tract of a new host, the first change of the spore prior to the germination of the sporoplasm is the extrusion of its polar filament, according to the observations made by Thélohan (1895), Stempell (1909), Fantham and Porter (1912), Kudo (1916), and others. Osmotic pressure is considered in this case to be the cause of the filament extrusion.

In the case of the artificial cultivation of the Cnidosporidia, starting with the spore-stage, therefore, the first observation should be concentrated on the filament. From this point of view I have again taken up the question of the filament-extrusion, which I partially worked out about four years ago (Kudo, 1913).

Since Balbiani discovered in some Myxosporidian spores the presence of a polar filament which was extruded from the spore under the action of alkalies, several investigators have tried a large number of reagents in the effort to produce extrusion of the filament of Cnidosporidian spores of various species. The reagents used and the authors' names are given in Table 1.

Most authors agree that the percentage of spores which extrude the filament when subjected to the action of the above-mentioned reagents is very small and irregular. It seems quite remarkable that these reagents should affect only certain particular species, as would appear from Thélohan's experiment (1895).

In the case of mechanical pressure, I could see almost all of the spores extruding their filaments at places where the pressure was apparently strong enough to drive the filament out. When the spore material is mixed with comparatively coarse particles of tissue, however, the pressure method does not give uniform results.

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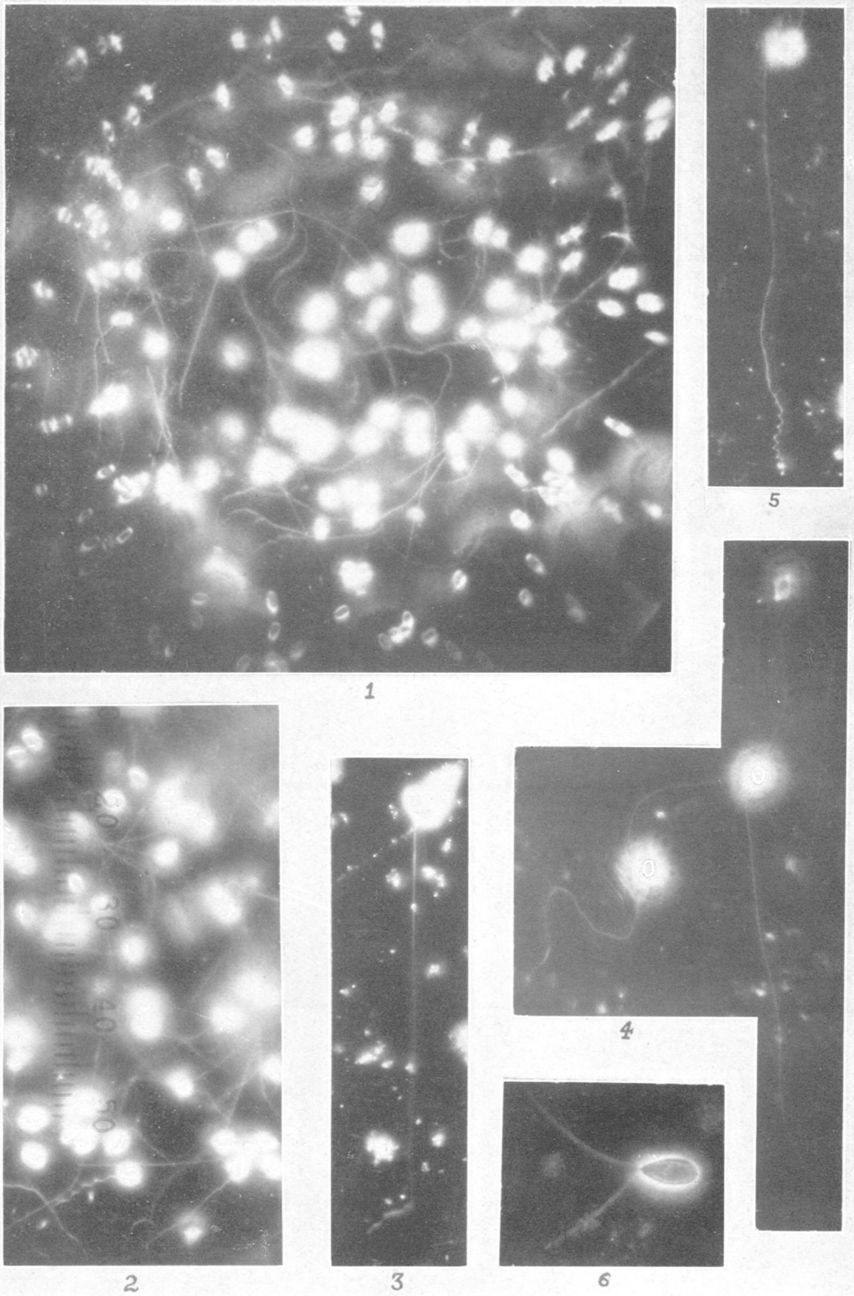
* Work carried out in the laboratories of Dr. Noguchi and under his direction.



Explanation of Plate

Figs. 1-5.—Spores of *Nosema bombycis* with extruded polar filament after subjection to the action of perhydrol.

Fig. 6.—A spore of *Myxosoma funduli* treated in the same way.

Frey and Lebert and Haberlandt and Verson studied the action of several chemicals upon the spore of *Nosema bombycis* without noticing the filament extrusion.

Of the many chemicals which I have tried up to the present, hydrogen peroxid, as first found by Dr. Noguchi, has proved best. Following is a brief summary of the experiments with this reagent, which have been carried out chiefly on *Nosema bombycis*.

TABLE 1

Reagents	Author's Name and Parasite Used
Acetic acid	Thélohan (*Myxosoma, Henneguya*), Wasielewski, Fantham and Porter (*Nosema apis*)
Ether	Thélohan (*Thelohania*) Gurley, Pfeiffer, Wasielewski, Auerbach
Alkalies (KOH and NaOH)	Balbiani, Thélohan, Wasielewski, Auerbach, Kudo
Ammonia	Auerbach, Schuberg (*Pleistophora longifilis*)
Boiling water	Gurley, Wasielewski, Auerbach
Distilled water	Thélohan (*Chloromyxum*), Wasielewski, Auerbach
Glycerin	Schneider, Gurley, Wasielewski, Auerbach, Awerinzew (*Myxobolus magnus*)
Iodin alcohol	Stempell (*Thelohania mülleri, Nosema anomalum*)
Iodin water	Thélohan (*Ceratomyxa, Glugea, Pleistophora*), Léger, Wasielewski, Auerbach, Fantham and Porter
Mineral acids:	
Hydrochloric acid	Thélohan (*Thelohania*), Gurley, Wasielewski, Auerbach
Nitric acid	Thélohan (*Myxospordia, Glugea bombycis, Thelohania*), Gurley, Pfeiffer (*Thelohania mülleri*), Wasielewski
Sulphuric acid	Bütschli, Thélohan (*Sphaeromyxa*), Gurley, Wasielewski, Auerbach, Awerinzew
Physiological solution	Lacépède (*Pleistophora macrospora*)
Pressure	Gurley, Auerbach, Kudo

The sterile silk-glands taken from the highly infected larvae of *Bombyx mori* furnished the original material for the experiment. They were emulsified with sterile water as well as with sterile Ringer's solution, kept in small test tubes in the refrigerator at a temperature of 6° C., and taken out from time to time for examination. An equal quantity of a strong solution of hydrogen peroxid and the emulsion were mixed either on a slide or in a test tube and examined microscopically. Observations were usually made under the dark field microscope, which, though it seems to have been neglected in this field of protozoology, affords a quick and entirely suitable means of carrying out the necessary observations. The permanent preparation was made according to Löffler—Kudo's method (1913). Fontana's staining for *Treponema pallidum,* however, was found to be equally good.

When a drop of the emulsion of *Nosema bombycis* is mixed on an acid-free slide with a drop of perhydrol,* active bubbling takes place. By examining the preparation under the dark-field microscope, almost all of the spores are seen to shoot out the polar filament with great rapidity (see plate). The phenomenon takes place immediately and lasts for several minutes.

The emulsion in Ringer's solution seems to give a more vigorous extrusion than the plain water emulsion.

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* About 30% H_2O_2 by weight, Merck.

A weak alkalin reaction of the emulsion as rendered by the addition of sodium bicarbonate seems to accelerate the extrusion of filaments, while acidulation inhibits extrusion.

That a concentrated solution of perhydrol is much more effective than a weaker one is shown in Table 4.

An attempt was made to induce the extrusion of polar filaments with the emulsions prepared from old dried materials, but it was found that no extrusion took place with such specimens. Apparently the desiccation of the spores rendered them insensitive to the extruding action of the reagent. The results of the experiments made with freshly dried materials were identical with those obtained with the old specimens and are shown in Table 5. An electric fan was employed to dry the emulsion during the first 24 hours, after which the slides with the dried emulsions were left at room temperature.

TABLE 2

	Immediately After Treatment	After One Hour	After 24 Hours
Water emulsion, 1 c.c. + perhydrol, 2 c.c.	Many extruded filaments	Fairly many extrusions	Many filaments detached
Ringer emulsion, 1 c.c., + perhydrol, 2 c.c.	Almost all spores show filament	Almost all spores show filament	Many filaments detached

TABLE 3

	Control	$NaHCO_3$ (1%)	HCl (1%)
Ringer emulsion, 1 c.c., + perhydrol, 2 c.c.	Many filaments extruded	Almost all spores show extruded filament	No extrusion

TABLE 4

Ringer emulsion, 1 c.c. + perhydrol (30%), 1 c.c.	Immediately after the treatment great many extruded filaments
Ringer emulsion, 1 c.c. + perhydrol solution (3%), 1 c.c.	A few extruded filaments

TABLE 5

	Perhydrol and spores in alkalinized emulsion	1% aqueous emulsion of the fresh tissue, $NaHCO_3$ and perhydrol	Spores taken from dried moths, which were kept over one year, pressure method
Immediately after the aqueous emulsion was made	Almost all of the spores show extruding filaments	Vigorous bubbling	Many filaments
Dried for 2 hours on slide	Fairly many extruded filaments	Bubbling less active	
Dried for 16 hours on slide	Many extruded filaments	Weak bubbling	
Dried for 24 hours on slide	A few detached filaments (?)	Only few bubbles	
Dried for 3 days on slide	No extrusion	No bubbling nor bubbles	
Dried for 5 days on slide	No extrusion	No bubbling nor bubbles	
Dried for 10 days on slide	No extrusion	No bubbling nor bubbles	

It is noteworthy that the number of extruded filaments progressively diminished as the desiccation went on, until, after three days, the phenomenon ceased to occur. With regard to the mechanism of extrusion by means of perhydrol, it seems probable that a physical force, comparable to that produced by pressure, develops within the spores and expels the polar filament. This force may be none other than the gas evolved through the decomposition of hydrogen peroxid by the peroxydase contained within the spores. In support of this view it may be cited that the spores preserved for more than one year still extruded their filaments when subjected to mechanical pressure. The failure of the dried spores, therefore, to extrude the polar filament under the action of perhydrol is a sign of the weakening or absence of the peroxydase, but bears no relation to the viability of such spores.

TABLE 6

	Perhydrol + spores in alkalinized emulsion	Pressure
1 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
3 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
10 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
30 per cent. methyl alcohol	Filament extrusion..........	Filament extrusion
60 per cent. methyl alcohol	Few extruded filaments....	Few extruded filaments
70 per cent. methyl alcohol	Few detached filaments (?).	?
85 per cent. methyl alcohol	No extrusion	No extrusion
Absolute methyl alcohol...	No extrusion	No extrusion
Control without alcohol treatment	Almost all spores show extruded filament	Many extruded filaments

TABLE 7

	Perhydrol + spores in alkalanized emulsion	Pressure
10% ethyl alcohol, 16 hours	Few extruded filaments.....	Many extruded filaments
30% ethyl alcohol, 16 hours	Few extruded filaments.....	Few extruded filaments
34% ethyl alcohol, 16 hours	Few extruded filaments.....	?
38% ethyl alcohol, 16 hours	No extruded filaments......	?
50% ethyl alcohol, 16 hours	No extruded filaments......	No extruded filaments
80% ethyl alcohol, 16 hours	No extruded filaments......	No extruded filametns
Absolute methyl alcohol...	No extruded filaments......	No extruded filametns
Control without alcohol treatment	Almost all spores show extruded filament	Extruded filaments in many places

TABLE 8

	Ethyl Alcohol		Methyl Alcohol	
	$+H_2O_2$ (0.02 C.c.)	$+1\%$ $NaHCO_3$ (0.5 C.c.) $+H_2O_2$ (0.02 C.c.)	$+H_2O_2$ (0.02 C.c.)	$+1\%$ $NaHCO_3$ (0.5 C.c.) $+H_2O_2$ (0.02 C.c.)
Control without alcoholic treatment	Vigorous bubbling	Vigorous bubbling	Vigorous bubbling	Vigorous bubbling
Emulsion +10%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +20%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +30%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +40%	Active bubbling	Active bubbling	Less active bubbling	Active bubbling
Emulsion +50%	Active bubbling	Active bubbling	Less active bubbling	Active bubbling
Emulsion +60%	Less active bubbling	Active bubbling	No bubbling	Less active bubbling
Emulsion +70%	Less active bubbling	Active bubbling	No bubbling	Much less active bubbling
Emulsion +80%	No bubbling	Less active bubbling	No bubbling	No bubbling
Emulsion +90%	No bubbling	No bubbling	No bubbling	No bubbling
Emulsion + absolute	No bubbling	No bubbling	No bubbling	No bubbling

It is evident that in the case of perhydrol, the extrusion of filaments is produced by a positive pressure created within the spores, but it was not shown whether or not a negative pressure by means of a vacuum will accomplish the same effect. For this reason, fresh Nosema spores were placed in a vacuum jar from which the air was exhausted with an electric vacuum pump. A vacuum at 2 mm. of mercury maintained for one hour did not expel the polar filaments from the spores.

With reference to the effect of alcohol upon the extrusion of polar filaments, it may be remarked here that Thélohan (1895) working with various chemicals as already quoted in the earlier part of this article, failed to obtain any extrusion with several Myxosporidia preserved in alcohol. On the other hand, Gurley (1894) reports that a rather small proportion of the spores of certain Myxosporidia preserved in alcohol extruded the polar filament under the action both of sulphuric acid and iodin water.

An experiment bearing on this point was made in the present study with *Nosema bombycis*. The technic used to test the action of alcohol upon the Nosema spores consisted in mixing 0.5 c.c. of a water emulsion and 3 c.c. of each of several alcohols in test tubes and allowing the mixtures to stand for ten minutes, inclusive of the time required for centrifugation, or, in some instances, for 16 hours at room temperature before centrifugation. The spores deposited at the bottom of the centrifuge tubes were subjected to the action of perhydrol in concentrated form. The results are recorded in Table 6 and 7.

As will be noted in the foregoing tables, of the spores subjected to the action of 60 per cent. methyl alcohol for 10 minutes, or of 34 per cent. ethyl alcohol for 16 hours, a few still extrude their polar filaments when treated with perhydrol. No polar filament is seen to be extruded from the spores treated with 85 per cent. methyl alcohol for 10 minutes or 30 per cent. ethyl alcohol for 16 hours. Methyl alcohol weaker than 30 per cent. allowed to act for 10 minutes had much less effect upon the phenomenon. Spores which have remained in 30 per cent. ethyl alcohol for sixteen hours were rendered almost insusceptible to the extruding action of perhydrol, and the filament extrusion became less as the percentage of alcohols rose, until, when the spores were treated with strong alcohol for a sufficient length of time, no filament escaped from the spore. In this instance, as in that of desiccation, the reduction of susceptibility of these spores to the extruding action of perhydrol may be explained by assumptions similar to those advanced for the effect of desiccation. Alcohol may have altered the permeability of the spores, or possibly the elasticity of the polar filament is reduced by the hardening action of alcohols, rendering it unable to unfold for protrusion. Another factor is the gradual destruction of the peroxydase as the concentration of alcohols is increased. Table 8 records the results of an experiment which shows that the peroxydase is considerably damaged by 30 to 40 per cent. methyl alcohol or 50 to 60 per cent. ethyl alcohol when acted upon for three hours. Thus 0.05 gm. of a fresh rabbit's kidney was emulsified with 5 c.c. of distilled water; 0.05 c.c. of the emulsion was mixed with 0.5 c.c. of each of several grades of alcohol, and the mixtures were left in the refrigerator at 6° C. for three hours. At the end of this period they were further treated with perhydrol (0.02 c.c.), or with 1 per cent. $NaHCO_3$ (0.05 c.c.) and perhydrol (0.02 c.c.). The results were as follows:

It may be concluded, therefore, that a fresh tissue treated with 90 per cent. ethyl alcohol or 80 per cent. methyl alcohol is no longer able to split hydrogen peroxid under the experimental conditions described above. Assuming that the extrusion of polar filaments is caused by the decomposition of H_2O_2 by a minute amount of the intracellular ferment (peroxydase), it is not difficult to understand why the extrusion becomes less certain when desiccation or alcohol treatment is applied to the spores, since there results an inactivation of the ferment sufficient to cause the occurrence of the phenomenon.

Spores of several species of Myxosporidia were also subjected to the action of perhydrol. The following Myxosporidian spores, according to my observations (Kudo, 1916a) when treated with this reagent, extrude their filaments: *Myxidium* sp.; *Zschokkeella acheilognathi* Kudo; *Myxosoma funduli* Kudo (Fig. 6).

SUMMARY

1. A concentrated solution of hydrogen peroxid is the most perfect and convenient reagent for producing extrusion of the polar filament from spores of *Nosema bombycis* and of some Myxosporidia in the fresh state.

2. The action of hydrogen peroxid is accelerated by the presence of weak alkalies.

3. Ringer's solution emulsion is more favorable for filament extrusion than water emulsion.

4. The action of hydrogen peroxid in extruding the polar filament is less effective upon spores which have been desiccated at room temperature than upon fresh ones. Spores dried on a slide for three days do not extrude the filament.

5. The pressure method gives, generally speaking, the same results as the perhydrol method, except that it produces fewer examples of extruded filament.

6. A spore emulsion centrifuged with 60 per cent. methyl alcohol for 10 minutes or mixed with 34 per cent. ethyl alcohol for 16 hours shows filament extrusion under the action of perhydrol.

REFERENCES CITED

Auerbach, M. 1910. Die Cnidosporidien. Leipzig.

Fantham, H. B., and Porter, Annie. 1912. The Morphology and Life History of Nosema apis and the significance of its various stages in the so-called "Isle of Wight" Disease in Bees (Microsporidiosis). Ann. Trop. Med. Parasit., 6: 163-196.

Gurley, R. R. 1894. The Myxosporidia, or Psorosperms of Fishes and the Epidemics Produced by them. Report, U. S. Fish Comm., 5: 65-304; 47 pl.

Kudo, R. 1913. Eine neue Methode die Sporen von Nosema bombycis Nägeli mit ihren ausgeschnellten Polfäden dauerhaft zu präparieren und deren Länge genauer zu bestimmen. Zool. Anz., 41: 368-371.

1916. Contributions to the Study of Parasitic Protozoa. I. On the Structure and Life-history of Nosema bombycis Nägeli. Bull. Imp. Sericultural Exp. Station, 1: 31-51.

1916a. Contributions to the Study of Parasitic Protozoa. III. Notes on Myxosporidia found in some Fresh-Water Fishes of Japan, with the Description of Three New Species. Jour. Parasit., 3: 3-9.

Stempell, W. 1909. Uber Nosema bombycis Nägeli. Arch. f. Protistenk., 16: 281-358.

Thélohan, P. 1895. Recherches sur les Myxosporidiens. Bull. scient. France et Belg., 26: 100-394.

Wasielewski, T. von. 1896. Die Sporozoenkunde. 166 pp. Jena.